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10/584,338	01/09/2007	Kevin Allen D'Amour	CYTHERA.045NP	3658

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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

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Office Action Summary	Application No.	Applicant(s)	
	10/584,338	D'AMOUR ET AL.	
	Examiner	Art Unit	
	WU-CHENG Winston SHEN	1632	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *12 May 2009*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *76-88* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *76-88* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *05/12/2009*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Applicant's claim amendments received on 05/12/2009 has been entered. The Declaration filed on 05/12/2009 by Dr. Kevin D'Amour has been entered and considered.

Claim 76 is amended. Claim 88 is newly added. Claims 76-88 are currently under examination.

This application 10/584,338 is a 371 of PCT/US04/43696 filed on 12/23/2004 which claims benefit of 60/532,004 filed on 12/23/2003, and claims benefit of 60/586,566 filed on 07/09/2004 and claims benefit of 60/587,942 filed on 07/14/2004.

Color Drawings

The Examiner notes that it has been acknowledged in the office action mailed on 02/12/2009 that the drawings filed on 06/23/2006 in black and white are accepted. However, it is noted that acceptance of color drawings requires petition by Applicant with information below.

Color photographs and color drawings are acceptable only for examination purposes unless a petition filed under 37 CFR 1.84(a)(2) is granted permitting their use as acceptable drawings. In the event that applicant wishes to use the drawings currently on file as acceptable drawings, a petition must be filed for acceptance of the color photographs or color drawings as acceptable drawings. Any such petition must be accompanied by the appropriate fee set forth in 37 CFR 1.17(h), three sets of color drawings or color photographs, as appropriate, and an amendment to the first paragraph of the brief description of the drawings section of the specification which states:

> The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the U.S. Patent and Trademark Office upon request and payment of the necessary fee.

Color photographs will be accepted if the conditions for accepting color drawings have been satisfied.

In the instant case, it appears that color drawings were filed with the instant application on 06/23/2006. Applicants are required to fulfill the requirements for color drawings as outlined above.

Claim Rejection - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

1. Claims 76-87 remain rejected and new claim 88 is rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a method of producing human definitive endoderm cells, said method comprising: obtaining a cell population comprising pluripotent human embryonic stem (hES) cells or human embryonic germ (hEG) cells; and providing said cell population with activin A and Wnt3a, providing serum at concentration lower than 0.2 μg/ml initially to said cell population, thereby generating in said cell population human definitive endoderm cells expressing at least SOX 17 and HNF3β, **does not** reasonably provide enablement of the said methods for (1) obtaining a cell population comprising induced pluripotent stem (iPS) for producing human definitive endoderm cells, (2) providing said cell population with any TGFβ superfamily growth factor in combination with any Wnt-pathway

activator other than the TGFβ superfamily growth factor activin A in combination with the Wnt-pathway activator Wnt3a, or (3) providing serum at any concentration for initial culture of pluripotent human cells other than providing serum at concentration lower than 0.2 μg/ml initially to said cell population. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.

Applicant's arguments filed 05/12/2009 have been fully considered and they are not persuasive. Previous rejection is ***maintained*** for the reasons of record advanced on pages 2-9 of the office action mailed on 02/12/2009.

It is noted that the aspect of rejection pertaining to non-enable embodiment for producing any mammalian species definitive endoderm cells other than human definitive endoderm cells is *withdrawn* because independent claim 76 filed on 05/12/2009 has been amended in this regard.

For the clarity and completeness of this office action, the rejection for the reasons of record advanced on pages 2-9 of the office action mailed on 02/12/2009, is reiterated below, with revisions addressing claim amendments filed on 05/12/009.

Enablement is considered in view of the Wands factors (MPEP 2164.01(a)). The court in Wands states: "Enablement is not precluded by the necessity for some experimentation such as routine screening. However, experimentation needed to practice the invention must not be undue experimentation. The key word is 'undue,' not 'experimentation.' " (*Wands*, 8 USPQ2d 1404). Clearly, enablement of a claimed invention cannot be predicated on the basis of quantity of experimentation required to make or use the invention. "Whether undue experimentation is needed is not a single, simple factual determination, but rather is a conclusion reached by weighing many factual considerations." (*Wands*, 8 USPQ2d 1404). The factors to be considered

in determining whether undue experimentation is required include: (1) the quantity of experimentation necessary, (2) the amount or direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the breadth of the claims. While all of these factors are considered, a sufficient amount for a *prima facie* case is discussed below.

The nature of the invention is directed to a method for producing human definitive endoderm by directed *in vitro* differentiation of cultured human embryonic stem (hES) cells in the presence of a TGFβ superfamily growth factor and a Wnt-pathway activator.

The breadth of the claims a method for producing a method of producing any definitive endoderm cells, said method comprising: obtaining a cell population comprising any pluripotent human cells; and providing said cell population with any member of TGFβ superfamily growth factor and any member of the Wnt-pathway activators, providing serum at any concentration initially to said cell population.

The specification discloses that human ES and EG cells (hESCs) offer unique opportunities for investigating early stages of human development as well as for therapeutic intervention in several disease states, such as diabetes mellitus and Parkinson's disease (See paragraph [0004] of instant application).

With regard to growth factors required for differentiation of hES cells, the specification discloses that in some embodiments of the present invention, one or more growth factors are used in the differentiation process from pluripotent cell to definitive endoderm cell. The one or more growth factors used in the differentiation process can include growth factors from the TGFβ superfamily. In such embodiments, the one or more growth factors comprise the

Nodal/Activin and/or the BMP subgroups of the TGFβ superfamily of growth factors. In some embodiments, the one or more growth factors are selected from the group consisting of Nodal, Activin A, Activin B, BMP4, Wnt3a or combinations of any of these growth factors (See paragraph [0010], [0215], and Example 6 of instant application).

With regard to markers of definitive endoderm cells, the specification discloses that in some embodiments, one or more markers selected from **SOX17**, CXCR4, MIXL1, GATA4, **HNF3β**, GSC, FGF17, VWF, CALCR, FOXQ1, CMKOR1 and CRIP1 are expressed in definitive endoderm cells. In other embodiments, one or more markers selected from OCT4, alpha-fetoprotein (AFP), Thrombomodulin (TM), SPARC and SOX7 are not significantly expressed in definitive endoderm cells (See paragraph [0008], Table 1, and Examples 6-11 of instant application).

The specification does not provide enabling support for **(1)** obtaining a cell population comprising induced pluripotent stem (iPS) for producing human definitive endoderm cells, **(2)** providing said cell population with any TGFβ superfamily growth factor in combination with any Wnt-pathway activator other than the TGFβ superfamily growth factor activin A in combination with the Wnt-pathway activator Wnt3a, or **(3)** providing serum at any concentration for initial culture of pluripotent human cells other than providing serum at concentration lower than 0.2 μg/ml initially to said cell population.

(1) Pertaining to the claimed pluripotent human cell population of claimed method, it is noted that the pluripotent human cell recited in claim 1 reads on induced pluripotent human stem cells (iPS), which have not been demonstrated to behave in an identical way as pluripotent

embryonic stem cells. Furthermore, the Examiner notes that the specification does not contemplate on using induced pluripotent stem cells (iPS) for the claimed method because the method for generation of induced pluripotent stem cells (iPS) was not substantiated until 2006, which is after the claimed priority date of instant application. In this regard, For instance, **Takahashi et al.** demonstrates the generation of iPS cells from adult human dermal fibroblasts with the expression of the same four factors: Oct3/4, Sox2, Klf4, and c-Myc. Takahashi et al. teaches that human iPS cells were similar to human embryonic stem (ES) cells in morphology, proliferation, surface antigens, gene expression, epigenetic status of pluripotent cell-specific genes, and telomerase activity, and furthermore, these cells could differentiate into cell types of the three germ layers *in vitro* and in teratomas (See summary, Takahashi et al., Induction of pluripotent stem cells from adult human fibroblasts by defined factors, *Cell* 131(5):861-72, 2007).

(2) With regard to growth factors required for differentiation of hES cells to human definitive endoderm cells, it is worth noting that members of TGFβ superfamily are involved in regulation of various signaling pathways. **Valdimarsdottir et al.** provides the following statements regarding the status of art pertaining to the unpredictable nature of *in vitro* differentiation of hES cells and the role of TGFβ superfamily signaling during differentiation: It is clear that as long as hES cell lines are not clonally (re-) derived and culture conditions differ substantially between laboratories with a resulting degree of selection, it will be difficult to make broad generalizations on the molecular basis of self-renewal and differentiation control. In fact, different lines may be suitable for different purposes. Nevertheless, as the literature grows, some universal principles are emerging that are common to mouse and human ES cells, suggesting that

transcriptional control may be conserved although the upstream pathways by which it is regulated may show species specificity. This emphasizes the importance of studying hES cell and mES cells in parallel whenever possible. In addition to their presumed importance in self-renewal, the TGFβ family members have been shown to have multiple regulatory roles in ES cell differentiation. TGFβ superfamily signaling can lead to very different effects, ranging from maintenance of self-renewal to specific differentiation steps. The decision that a cell makes when subjected to a given cytokine might therefore depend on the particular state of the cell upon receiving the signal and on the ligand concentration (See Conclusion, left column, page 784, Valdimarsdottir et al., Functions of the TGFβ superfamily in human embryonic stem cells. *APMIS*, 113(11-12):773-89, 2005). With regard to activation of Wnt pathway for differentiation of hES cells, **Ciani et al.** teaches that Wnt signaling has a key role in early embryonic patterning through the regulation of cell fate decisions, tissue polarity and cell movements, and that there are three main branches of the Wnt signaling pathway that regulate distinct sets of gene expression (See abstract and Figure 1, Ciani et al., WNTs in the vertebrate nervous system: from patterning to neuronal connectivity, *Nat Rev Neurosci.* 6(5):351-62, 2005). Therefore, in the absence of indication of specific recitation requiring combination of activin A and Wnt3a to initiate hES cells differentiation in the claimed methods, one of skill in the art would have to perform undue experimentation to make and use the claimed invention commensurate in scope with the claims 76-88. This is of particular importance in light of the unpredictability in the art regarding *in vitro* differentiation of hES cells and the fine tuned roles of TGFβ superfamily signaling taught by Valdimarsdottir et al. and the presence of multiple and distinct Wnt signaling pathways taught by Ciani et al.

(3) Pertaining to specific requirement for *in vitro* differentiation of hES cells, the state of the art indicates that the presence of insulin in the medium suppresses definitive endoderm differentiation from human embryonic stem cells. For instance **McLean et al.** reported that contacting human embryonic stem cell with insulin in a concentration as little as 0.2 μg/ml during the differentiation process is detrimental to the production of definitive endoderm (See Fig. 6, Mclean et al., Activin A efficiently specifies definitive endoderm from human embryonic stem cells only when phosphatidylinositol 3-kinase signaling is suppressed, *Stem Cells*, 25(1):29-38, 2007).

As issues pertaining to *in vitro* differentiation of hES cells discussed above, in view of the state of the art, the unpredictability in the art, and the lack of specific guidance and working examples in the specification, one of skill in the art would have to perform undue experimentation to make and use the claimed invention commensurate in scope with the claims 76-88.

Applicant's arguments and Examiner's ***Response to Applicant's arguments***

(1) With regard to the pluripotent human cells recited in claim 1 reads on induced pluripotent stem cells (iPS), Applicant argues that Yu et al. (Human Induced Pluripotent Stem Cells Free of Vector and Transgene Sequences, *Science*, 324:797-801 (2009)) demonstrate that iPS cells possess the same features as human ES cells that distinguish them from other cell types. Applicant argues that, furthermore, Yu et al. demonstrate that iPS cells can differentiate into cell types of the three germs layers both in vitro and in vivo. Specifically, Yu et al., states:

These iPS cell colonies exhibited typical human ES cell morphology (e.g., compact colonies, high nucleus-to-cytoplasm ratios, and prominent nucleoli) (Fig. 1 B) and exhibited

gene expression profiles that were very similar to those of the parental fibroblasts (Fig. 1C and table $3). Similar to human ES cells, when injected into immunocompromised mice, these iPS cells formed teratomas consisting of differentiated derivatives of all three primary, germ layers (Fig. 1D). ... The iPS cell subclones were morphologically similar to human ES cells (Fig. 3A); had normal karyotypes (Fig. 3B); expressed human ES cell-specific cell surface markers (Fig. 3D) and genes (Fig. 4, A and B, fig. $4, and table $4); and differentiated into derivatives of all three germ layers in teratomas (Fig. 4C) (emphasis added; Yu et al. at 799-800)

Applicant argues that Yu et al. also demonstrate that iPS cells "behave in an identical way to pluripotent stem cells" as indicated in the claims. Also, Zhang et al. demonstrate that iPS cells can be differentiated into insulin-producing cells using the same step-wise protocol used for hES cells. Zhang et al., Highly Efficient Differentiation of Human ES Cells and iPS Cells into Mature Pancreatic Insulin- Producing Cells. *Cell Research* (2009) 19:429-438 (enclosed herewith for the Examiner's convenience).

With regard to total absence of disclosure in the specification of the instant application contemplates pertaining to the use of iPS cells as a species of pluripotent cells that can be used to practice the claimed method, Applicant states that it is well-established that an inventor need not describe every potential type of material that can be utilized in a claimed method. Applicant argues that the fact that later developed materials can be used in connection with a claimed process without undue experimentation is well reflected in section 2164.08 of the MPEP as well as current case law. Applicant argues that the proper inquiry is not whether the material existed at the time the specification was filed, but rather, whether a skilled artisan would be able to perform the steps of the method on the material so as to achieve the stated result without undue experimentation. Applicant argues that as discussed above, human iPS cells have been shown to

behave just as human ES cells in their ability to differentiate. Furthermore, Applicant argues that human iPS cells have been shown to be responsive to signaling factors in the same way as human ES cells. As such, it is clear that a skilled artisan could practice the claimed method using iPS cells without undue experimentation.

In response: Applicant's arguments filed 05/12/2009 have been fully considered and they are not persuasive. The Examiner notes that the disclosure of instant application does not constitute the *conception of the invention* pertaining to human iPS cells because as stated in the maintained rejection, the specification does not contemplate on using induced pluripotent stem cells (iPS) for the claimed method as the specification does not disclose anything in this regard. In fact, the method for generation of induced pluripotent stem cells (iPS) was not substantiated until 2006, which is after the claimed priority date of instant application. It is worth noting that the methods for creating induced pluripotent stem cells (iPS) were realized in 2006 by groups of researchers led by James A. Thomson and by Shinya Yamanaka, which are not inventors of instant application. There is no evidence of record in either stem cell literature or specification of instant application supports that the inventors of instant application have the *conception of the invention* pertaining to human iPS cells can be used for claimed method of producing human definitive endoderm cells. Moreover, it is noted that there must be a *contemporaneous recognition and appreciation* of the invention for there to be conception. Silvestri v. Grant, 496 F.2d 593, 596, 181 USPQ 706, 708 (CCPA 1974). Furthermore, "*conception is not enablement*," conception of a purified DNA sequence coding for a specific protein by function and a method for its isolation that could be carried out by one of ordinary skill in the art is not conception of

that material). MPEP 2138.04. The Examiner emphasizes that, at the time of priority date of instant application (12/23/2003), there was no contemporaneous recognition and appreciation regarding a method of producing human definitive endoderm cells from human iPS cells. In fact, dated back to 2003, it was not even clear whether a differentiated human cell can be induced (or de-differentiated) to become induced pluripotent human stem cells (i.e. human iPS cells) and what defining characteristics human iPS cells should have.

Applicant only relies on the cited post-filing art in 2009 to provide enabling support of the claimed invention of instant application. In the absence of clearly documented conception of invention at the time of filing (filing date of instant application 01/09/2007, with priority dated back to 12/23/2003), lack of specific guidance, no working example provided, it will take undue experimentation for a skilled person in the art to make and use the claimed invention commensurate in scope with the claims 76-88. Therefore, the post-filing art fails to overcome the lack of guidance in the specification and the unpredictability of the art at the time of filing. Knowledge gained and disclosed in the post-filing art that was not available at the time the invention was made, fails to offer enabling support to the claims. Accordingly, this aspect of the scope of enablement rejection of claims 76-88 that the pluripotent human cells recited in claim 1 reads induced pluripotent stem cells (iPS) under 35 U.S.C. 112, first paragraph, is maintained of the record.

(2). With regard to providing TGFβ superfamily growth factors for differentiation of hES cells to human definitive endoderm cells, Applicant states that currently, there are approximately 33 agents known to interact with a TGF-β receptor family member. Applicants files a

Declaration under 37 C.F.R. § 1.132 by Dr. Kevin D'Amour, which demonstrates that activin B, GDF8, and GDF11 are three additional agents capable of inducing definitive endoderm cells from human embryonic stem cells as recited in independent claim 76. Thus, Applicant states that Applicants have shown that at least 4 agents can activate a TGF-β receptor family member and induce differentiation of hES cells to human definitive endoderm cells. Furthermore, Applicant states that this declaration demonstrates that TGF-β 1, TGF-β 2, TGF-β 3, GDF3, GDF9 and GDF 15 are not capable of activating a TGF-β receptor family member so as to initiate differentiation of human embryonic stem cells to human definitive endoderm cells under the claimed conditions. As such, Applicant argues that Applicants have tested nearly a third of the known TGF-β superfamily growth factors and have demonstrated that about half of the factors tested promote the differentiation of hES cells to human definitive endoderm cells. The remaining TGF-β superfamily growth factors can easily be tested by a skilled artisan without undue experimentation in view of the guidance provided in the specification. Applicant argues that a claim does not lack enablement simply because it encompasses non-working embodiments (see section 2164.08(b) of the MPEP). Rather, "[t]he standard is whether a skilled person could determine which embodiments that were conceived, but not yet made, would be inoperative or operative with expenditure of no more effort than is normally required in the art." Atlas Powder Co. v. E.I. du Pont de Nemours & Co., 750 F.2d 1569, 1577 (Fed. Cir. 1984).

In response, Applicant's arguments and the Declaration filed by Dr. Kevin D'Amour have been fully considered and found not persuasive.

The following statements has been documented on pages 7-8 of the office action mailed on 02/12/2009 with revisions to address claim amendments filed on 05/12/2009.

Members of TGFβ superfamily are involved in regulation of various signaling pathways. Valdimarsdottir et al. provides the following statements regarding the status of art pertaining to the unpredictable nature of *in vitro* differentiation of hES cells and the role of TGFβ superfamily signaling during differentiation: It is clear that as long as hES cell lines are not clonally (re-) derived and culture conditions differ substantially between laboratories with a resulting degree of selection, it will be difficult to make broad generalizations on the molecular basis of self-renewal and differentiation control. In fact, different lines may be suitable for different purposes. Nevertheless, as the literature grows, some universal principles are emerging that are common to mouse and human ES cells, suggesting that transcriptional control may be conserved although the upstream pathways by which it is regulated may show species specificity. This emphasizes the importance of studying hES cell and mES cells in parallel whenever possible. In addition to their presumed importance in self-renewal, the TGFβ family members have been shown to have multiple regulatory roles in ES cell differentiation. TGFβ superfamily signaling can lead to very different effects, ranging from maintenance of self-renewal to specific differentiation steps. The decision that a cell makes when subjected to a given cytokine might therefore depend on the particular state of the cell upon receiving the signal and on the ligand concentration (See Conclusion, left column, page 784, Valdimarsdottir et al., Functions of the TGFβ superfamily in human embryonic stem cells. *APMIS*, 113(11-12):773-89, 2005). With regard to activation of Wnt pathway for differentiation of hES cells, **Ciani et al.** teaches that Wnt signaling has a key role in early embryonic patterning through the regulation of cell fate decisions, tissue polarity

and cell movements, and that there are three main branches of the Wnt signaling pathway that regulate distinct sets of gene expression (See abstract and Figure 1, Ciani et al., WNTs in the vertebrate nervous system: from patterning to neuronal connectivity, *Nat Rev Neurosci.* 6(5):351-62, 2005). Therefore, in the absence of indication of specific recitation requiring combination of activin A and Wnt3a to initiate hES cells differentiation in the claimed methods, one of skill in the art would have to perform undue experimentation to make and use the claimed invention commensurate in scope with the claims 76-88. This is of particular importance in light of the unpredictability in the art regarding *in vitro* differentiation of hES cells and the fine tuned roles of TGFβ superfamily signaling taught by Valdimarsdottir et al. and the presence of multiple and distinct Wnt signaling pathways taught by Ciani et al.

The Examiner acknowledges that the Declaration filed by Dr. Kevin D'Amour does provide additional information that is not disclosed in the specification of instant application. In this regard, the Declaration documents that activin B, GDF8, GDF11 of TGFβ superfamily, in addition to activin A disclosed in the specification, can induce the expression of SOX17 and FOXA2 expression, but lack induction of SOX7, which indicates the differentiation of hES cells to definitive endoderm proceeded through a mesendoderm intermediate as evidenced by the transient brahyury expression witnessed at 24 hours (See page 2, #6 of the Declaration).

However, it is noted that the claimed invention is directed to *in vitro* generation of human endoderm cells by providing pluripotent human cells population with a TGFβ superfamily growth factor and a Wnt-pathway activator, which is different from the conditions tested in the Declaration with a given TGFβ superfamily in the absence of a Wnt-pathway activator. Valdimarsdottir et al. clearly states that "the TGFβ family members have been shown to have

multiple regulatory roles in ES cell differentiation. TGFβ superfamily signaling can lead to very different effects, ranging from maintenance of self-renewal to specific differentiation steps". It is unpredictable whether the conditions for producing human definitive endoderm obtained from providing the TGFβ superfamily growth factor activin A in combination with the Wnt-pathway activator Wnt3a, as disclosed in the specification of instant application, can be directly applied to the definitive endoderm cells obtained from treating hES cells with any combination of any member of TGFβ superfamily with any Wnt-pathway activator. Dissecting the effects of each factor of the TGFβ superfamily on the intertwined signaling pathways involved in differentiation of hES cells to definitive endoderm in the presence of any Wnt-pathway activator, as claimed, is not considered as a routine experimentation as Applicant argues. To further demonstrate the unpredictability pertaining to signaling pathways regulated by TGFβ superfamily, **Saarma et al.** teaches that glial cell line-derived neurotrophic factor (GDNF) family, consisting of GDNF, neurturin, artemin and persephin are distant members of the transforming growth factor-beta (TGF-beta) superfamily, and unlike other members of the TGF-β superfamily, which signal through the receptor serine-threonine kinases, GDNF family ligands activate intracellular signalling cascades via the receptor tyrosine kinase Ret. (See abstract, Saarma et al., GDNF - a stranger in the TGF-β superfamily? *Eur J Biochem.* 267(24):6968-71, 2000). Taken together, it is unpredictable how a given member of TGFβ superfamily, including activin B, GDF8, GDF11 tested in the Declaration, will either activate or inhibit any of the downstream signaling pathways medicated by a given member of TGFβ superfamily in the same manner as combine effect of activin A and Wnt3a would have on differentiation of hES cells. Therefore, Applicant's arguments and the Declaration filed by Dr. Kevin D'Amour can not overcome the

unpredictability in the art for the reasons of record advanced on pages 7-8 of the office action mailed on 02/12/2009, and further elaboration provided in this office action.

(3) With regard to the unpredictability of providing serum at any concentration for initial culture of pluripotent human cells other than providing serum at concentration lower than 0.2 μg/ml initially to said cell population, Applicant states that McLean et al. point out that contacting human embryonic stem cells with insulin in a concentration as little as 0.2 μg/ml during the differentiation process is detrimental to the production of definitive endoderm. Applicant argues that McLean et al. do not, however, state that the presence of insulin would eliminate the differentiation of pluripotent cells to human definitive endoderm. Applicant argues that claim 76 merely lays out a method for the differentiation of pluripotent cells to human definitive endoderm and does not recite the efficiency of the differentiation process or a requisite number of definitive endoderm cells produced by the claimed method. Applicants argues that even if the practice of the method detailed in claim 76 resulted in the differentiation of only a single pluripotent cell to definitive endoderm, the claim would still be fully enabled.

In response, as discussed in **(2)**, the effect on differentiation of hES cells by various combinations of conditions encompassed by the limitation "providing pluripotent human cells population with a TGFβ superfamily growth factor and a Wnt-pathway activator" is unpredictable. Adding additional layer of detrimental effect of insulin at concentration higher than 0.2 μg/ml would certainly render the claimed method even more unpredictable in term of any human definitive endoderm cells can be successfully differentiated from recited steps.

Furthermore, Applicant's assertion that "even if the practice of the method detailed in claim 76 resulted in the differentiation of only a single pluripotent cell to definitive endoderm, the claim would still be fully enabled" appears to be in direct contradiction with the limitation "a method of producing human definitive endoderm cells" recited in the preamble of claim 76 and "said cell population definitive endoderm cells expressing at least SOX17 and HNF3b" recited in the step of claim 76. As a relevant issue in this regard, claim 76 does not recite any expansion step when a definitive endoderm cell is differentiated from a single pluripotent cell.

Claim Rejection - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

A person shall be entitled to a patent unless -

(e) the invention was described in (1) an application for patent, published under section 122(b), by another filed in the United States before the invention by the applicant for patent or (2) a patent granted on an application for patent by another filed in the United States before the invention by the applicant for patent, except that an international application filed under the treaty defined in section 351(a) shall have the effects for purposes of this subsection of an application filed in the United States only if the international application designated the United States and was published under Article 21(2) of such treaty in the English language.

2. Claims 76, 77, 79, 80, and 82-87 remain rejected under 35 U.S.C. 102(e) as being anticipated by **Fisk et al.** (U.S. Patent 7,326,572, issued 02/05/2008) as evidenced by **Kuo et al.** (Kuo et al., Roles of histone acetyltransferases and deacetylases in gene regulation *Bioessays*, 20(8):615-26, 1998). Applicant's arguments filed 05/12/2009 have been fully considered and they are not persuasive. Previous rejection is ***maintained*** for the reasons of record advanced on pages 9-11 of the office action mailed on 02/12/2009.

For the clarity and completeness of this office action, the rejection for the reasons of record advanced on pages 9-11 of the office action mailed on 02/12/2009, is reiterated below, with revisions addressing claim amendments filed on 05/12/009.

Claim 76 is directed to a method of producing definitive endoderm cells, said method comprising: obtaining a cell population comprising pluripotent human cells; and providing said cell population with a TGFβ superfamily growth factor and a Wnt- pathway activator, thereby generating in said cell population definitive endoderm cells expressing at least SOX 17 and HNF3β.

Claim interpretation: The limitation "a Wnt- pathway activator" recited in claim 76 reads on any agent or factor that positively affects, directly or indirectly, the expression of any gene involved in regulation of signaling activity of any component of a given Wnt-pathway in a transit or constitutive manner. This interpretation is consistent with (i) claim 81, which further limits claim 76 by "wherein said Wnt pathway activator is Wnt3a, and (ii) newly added claim 88, which further limits claim 76 by "wherein said Wnt-pathway activator is a Wnt family member".

With regard to the limitations of claims 76, 79, 80, 82, 83, 85, and 86 of instant application, Fisk et al. teaches endoderm derived from human embryonic stem cells (See title, Fisk et al.). Fisk et al teaches a method for generating endoderm cells from human embryonic stem (hES) cells or human embryonic germ (hEG) cells, comprising culturing the hES cells or hEG cells in a medium comprising a sufficient amount of Activin A (See Table 1, Fisk et al., which reads on claims 79, 80, 82 and 83 of instant application) to cause differentiation of said cells into endoderm, thereby generating endoderm cells, wherein the endoderm cells express the markers Sox 17, HNF3β and HNF4α (claim 1 of Fisk et al., which reads on definitive endoderm

cells expressing at least SOX 17 and HNF3β recited in claim 1 of instant application), wherein the medium further comprises sodium butyrate (claim 2 of Fisk et al., which reads on a Wnt-pathway activator recited in claim 1 of instant application), wherein the cells are human embryonic stem cells (claim 3 of Fisk et al., which reads on pluripotent human cells recited in claim 1 of instant application, and hES cells recited in claims 85 and 86 of instant application), wherein the cells are grown to confluence under feeder-free conditions before the hPS cells are cultured with Activin A (claim 4 of Fisk et al., which reads on "a TGFβ superfamily growth factor" recited in claim 76 of instant application). Fisk discloses that Sox 17 is a marker for identification of definitive endoderm cells and HNF3β is a marker for identification of endoderm cells (See Table 2 of Fisk et al.).

It is noted that Fisk et al. discloses n-butyrate (for instance, sodium butyrate) is a histone deacetylase (HDAC) inhibitor (See Table 1, Fisk et al.). Inhibition of HDAC leading to high acetylation level of histone during chromatin remodeling is known to activate transcription of genes in mammalian genome. Accordingly, n-butyrate is a non-ligand/receptor mediated signal-specific Wnt-pathway activator. The connection between high histone acetylation level and transcription activation is evidenced by **Kuo et al.** (See abstract and Figure 2, Kuo et al., Roles of histone acetyltransferases and deacetylases in gene regulation *Bioessays*, 20(8):615-26, 1998). It is worth noting that there is no requirement in the claim 76 of instant application that "a Wnt-pathway activator" be a protein growth factor.

With regard to removal of TGFβ superfamily growth factor from said cell population (claim 77 of instant application), Fisk et al. teaches step-wise differentiation steps of hES cells in which activin A is added in the initial media (See column 10-11, and Table 1) to maintain the

characteristics of pluripotent hES cells, and at later stages of differentiation the media no longer contains activin A (See for instance, Table 3, Fisk et al.).

With regard to the limitation "providing serum to said cell population in increasing concentration" (claim 84 of instant application), the broad and reasonable interpretation of this limitation reads on switching the old medium from one stage of differentiation to a new media of the next stage of differentiation, for instance Step II to Step III listed in Table 3 of Fisk et al., because fresh media would contain more serum compared to the media that have cells grown in for several days.

With regard the source of hES cells (claim 87 of instant application), Fisk et al. teaches that inner cell mass of embryo is one source of hES cells (See lines 13-29, column 8, Fisk et al.)

Thus, Fisk et al. (U.S. Patent 7,326,572, issued 02/05/2008) as evidenced by Kuo et al. (1998) clearly anticipates claims 76, 77, 79, 80, 82-87 of the instant application.

Applicant's arguments and Examiner's ***Response to Applicant's arguments***

Applicant argues that it is clear that **inherency may not be established by probabilities or possibilities.** Scaltech v. Retec/Tetra, L.L.C., 178 F.3d 1378 (Fed Cir. 1999), emphasis added. Applicant states that the mere fact that a certain thing may result from a given set of circumstances is not sufficient to establish inherency, Id. (citations omitted). Applicant states that the Examiner contends that inhibition of histone deacetylase by n-butyrate is known to activate transcription of genes in mammalian genome. Applicant argues that this does not establish that n-butyrate would activate the Wnt pathway. It is mere speculation that the general

activation of transcription of genes in the mammalian genome could potentially result in heightened activation of the Wnt pathway. Applicant argues that using this logic, it is also possible that general activation of the mammalian genome could just as well result in transcription of a factor that inhibits the Wnt pathway.

In response, the Examiner agrees with Applicant's arguments that general activation of the mammalian genome could just as well result in transcription of a factor that inhibits the Wnt pathway. In this regard, it is well-documented that regulation of transcription is a coordinated effect of multiple factors that can either positively or negatively influence final transcription output. Nevertheless, as indicated in the claim interpretation documented in this maintained rejection, the limitation "a Wnt-pathway activator", broadly reads on any agent or factor that positively affects, directly or indirectly, the expression of any gene involved in regulation of signaling activity of any component of a given Wnt-pathway in a transit or constitutive manner. Therefore, inhibition of histone deacetylase by n-butyrate to activate transcription of genes in mammalian genome inherently results in a positive effect, directly or indirectly, on the expression of any gene and signaling activity of any component of a given Wnt-pathway in a transit or constitutive manner. N-butyrate is recognized by the art as a Wnt pathway activator (Bordonard et al, Cell type- and promoter-dependent modulation of the Wnt signaling pathway by sodium butyrate, Int. J Cancer, 97(1):42-51, 2002). Thus, there is no probability, only definiteness in the role of N-butyrate in Wnt pathway. In this regard, it is worth noting that there are at least three known WNT pathways mediated through multiple distinct kinases, which in turn regulate expression of groups of genes involved in various cellular functions including cell

fate decision, cell and tissue polarity and cell movement (See for instance, Figure 1, Ciani et al., WNTs in the vertebrate nervous system: from patterning to neuronal connectivity, *Nat Rev Neurosci.* 6(5):351-62, 2005; This reference has been cited in the office action mailed on 02/12/2009). Therefore, based on the breadth of limitation of "a Wnt-pathway activator" interpreted and discussed in this maintained rejection, the biological activity of n-butyrate is inherently a non-ligand/receptor mediated signal-specific Wnt-pathway activator. As a related issue, it is noted that activin A is recognized by the art as a member of TGFβ superfamily (Thissese et al., Antivin, a novel and divergent member of the TGFβ superfamily, negatively regulates mesoderm induction, *Development*, 126(2):229-40, 1999). Thus, there is no probability, only definiteness of actvin A being a TGFβ superfamily growth factor. Applicant's attention is further directed to a relevant case law, *In re* Kubin, which demonstrates court decision that prior art disclosure of NAIL inherently contains disclosure of CD48 binding because binding to CD48 is an inherent biological activity of NAIL.

3. The following information has been documented on pages 11-12 of the office action mailed on 02/12/2009.

Additional references listed below are related to the claimed inventions.

(1). Fisk et al. US 7,033,831, issued on 04/25/2006

(2). Keller et al., US patent application publication 2006/0003446, publication date 01/05/2006.

(3) Prosecutions of Application 11/021,618 (US 2005/0158853) and Application 12/167,227 (US 2008/0267926).

Conclusion

4. **THIS ACTION IS MADE FINAL.** Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of this final action.

5. No claim is allowed.

Applicant is reminded that upon the cancellation of claims to a non-elected invention, the inventorship must be amended in compliance with 37 CFR 1.48(b) if one or more of the currently named inventors is no longer an inventor of at least one claim remaining in the application. Any amendment of inventorship must be accompanied by a request under 37 CFR 1.48(b) and by the fee required under 37 CFR 1.17(i).

Any inquiry concerning this communication from the examiner should be directed to Wu-Cheng Winston Shen whose telephone number is (571) 272-3157 and Fax number is 571-273-

3157. The examiner can normally be reached on Monday through Friday from 8:00 AM to 4:30 PM. If attempts to reach the examiner by telephone are unsuccessful, the supervisory patent examiner, Peter Paras, Jr. can be reached on (571) 272-4517. The fax number for TC 1600 is (571) 273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Deborah Crouch/
Primary Examiner, Art Unit 1632

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Patent Examiner
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